 Doi: 10.1111/j.1365-3059.2009.02152.x

Canopy-microclimate effects on the antagonism between *Trichoderma stromaticum* and *Moniliophthora perniciosa* in shaded cacao

L. L. Loguercio[a]*, L. S. Santos[a], G. R. Niella[b], R. A. C. Miranda[c], J. T. de Souza[d], R. T. Collins[e] and A.W.V. Pomella[f]†

[a]*Dept. Ciências Biológicas (DCB), Universidade Estadual de Santa Cruz (UESC), Rod. BR 415, Km 16, Ilhéus-BA, 45662-000;* [b]*Centro de Pesquisas do Cacau (CEPEC/CEPLAC), Rod. BR 415, Km 22, Cx. Postal 07, Ilhéus-BA, 45600-970;* [c]*Instituto de Geociências, Universidade Estadual do Rio de Janeiro (UERJ), Rua São Francisco Xavier 524, Sala 4007 - Bloco F. Maracanã-RJ, 20550-013;* [d]*Universidade Federal do Recôncavo da Bahia (UFRB), Centro de Ciências Agrárias Biológicas e Ambientais (CCABA), Cruz das Almas-BA, 44380-000, Brazil;* [e]*USDA-ARS, Sustainable Perennial Crops Laboratory, BARC-West, Beltsville-MD, 20705, USA; and* [f]*Almirante Cacau Ltda, Fazenda Almirante, BR 101 p/ Barro Preto Km 2, Cx. Postal 55, Itajuípe-BA, 45630-000, Brazil*

The collective impact of several environmental factors on the biocontrol activity of *Trichoderma stromaticum* (*Ts*) against *Moniliophthora perniciosa* (*Mp*), the cause of cacao witches' broom disease, was assessed under field conditions of shaded cacao (*Theobroma cacao*) in south-eastern Bahia, Brazil. Biocontrol experiments were performed adjacent to an automated weather station, with sensors and *Ts*-treated brooms placed at different canopy heights. Sporulation occurred at the same dates for all *Ts* isolates, but in different quantities. Broom moisture >30%, air temperature of approximately 23 ± 3°C, relative humidity >90%, solar radiation intensities <0·12 KW m^{-2} and wind speed near zero were the key environmental parameters that preceded *Ts* sporulation events. A multiple logistic regression indicated that these weather variables combined were capable of distinguishing sporulation from non-sporulation events, with a significant effect of wind speed. Analyses of environmental factors at ground level indicated similar pre-sporulation conditions, with a soil moisture content above a threshold of 0·34 m^3 m^{-3} preceding all sporulation events. The sporulation of five selected *Ts* isolates was compared at four different canopy heights. Isolates responded differently to weather variation in terms of sporulation and antagonism to *Mp* at different canopy levels, indicating that different microclimates are established along the vertical profile of a shaded cacao plantation. The potential of these findings for development of predictive mathematical models and disease-management approaches is discussed.

Keywords: antagonistic interaction, biological control, environmental factors, logistic regression, *Moniliophthora perniciosa*, *Theobroma cacao*

Introduction

Cacao (*Theobroma cacao*) has a significant social, economic and environmental relevance in south-eastern Bahia state (Brazil). Beyond its value for the chocolate and cosmetics industries, this crop constitutes a pragmatic paradigm for conservation of the threatened biodiversity of the Atlantic Rainforest. Cacao is grown in a system that preserves several native species for shading (Johns, 1999; Donald, 2004), thereby creating potentially functional habitats for forest species (Faria *et al.*, 2006). The outbreak in 1989 and rapid dissemination of witches' broom disease (Pereira *et al.*, 1996; Andebhran *et al.*, 1999), caused by the hemibiotrophic basidiomycete pathogen *Moniliophthora perniciosa* (Aime & Phillips-Mora, 2005), has resulted in major economic losses for the region, generating various social and environmental problems (Lopes *et al.*, 2003). Among these issues, the change in the prevalent economical activity in areas of shade-grown cacao plantations, with concomitant destruction of Atlantic Rainforest remnants, is critical from the conservation standpoint (Johns, 1999). The control of *M. perniciosa* (*Mp*) has included several methods, including phytosanitation, protective fungicide application (Pereira *et al.*, 1996; Purdy & Schmidt, 1996) and genetic resistance of bred clonal genotypes (Lopes *et al.*, 2003). However, because of practical and/or economical limitations of these strategies, emphasis has been given

*E-mail: leandro@uesc.br
†Current address: Laboratório de Biocontrole Farroupilha, Av. Cica nº 555, Patos de Minas-MG, 38702-054, Brazil.

to integrated disease management (Costa *et al.*, 2006), a scenario in which biological control has a prominent role. As the removal of dead brooms is one the most expensive practices in cocoa farming for control of witches' broom (Pomella *et al.*, 2007), 'biological broom pruning', by treating the cacao canopy with an efficient biocontrol agent, is a desirable objective.

Cosmopolitan occurrence, genetic and ecological diversity, host range and specificities, and synergistic mechanisms of antagonism are characteristics of *Trichoderma* species that contribute to their wide use as biocontrol agents (BCAs) of fungal plant diseases (Howell, 1998; Steyaert *et al.*, 2003; Benítez *et al.*, 2004). An isolate from the Amazon region, described as *Trichoderma stromaticum* (Samuels *et al.*, 2000), was introduced into south-eastern Bahia for specific mycoparasitic control of *Mp* (Bastos, 1996, 2000). Since 1999, spores of this isolate have formed the basis of the commercial preparation Tricovab (CEPLAC), which has given promising results for the management of witches' broom, prompting research efforts to improve its production and biocontrol efficiency (Pomella *et al.*, 2007; Loguercio *et al.*, 2009). Recent studies showed that *T. stromaticum* (*Ts*) is naturally widespread in south-eastern Bahia, displaying two major genetic groups (I and II; de Souza *et al.*, 2006) and a high variation in antagonistic ability of different *Ts* isolates was observed, mostly because of a remarkable effect of variation in environmental conditions (Santos, 2005; Carvalho, 2006).

The effects of environmental factors on survival, growth, reproduction, abundance and biocontrol activity of BCAs under laboratory and field conditions have been investigated (e.g. Dik & Elad, 1999; Sanogo *et al.*, 2002; Kredics *et al.*, 2000, 2004; Ten Hoopen *et al.*, 2003). Changing environmental conditions can have a significant impact on hyperparasitic relationships, with the final outcome being determined by specific weather patterns (Colhoun, 1973; Coakley, 1988). Hence, elucidating the interconnected effects of multiple meteorological factors may provide a better understanding of field inconsistencies in biocontrol, as well as supply more precise inputs for mathematical modelling aimed at simulating weather–biocontrol variations.

Hyperparasitism and plant infection are similar processes, as they require conditions that facilitate fungal spore germination, substrate invasion and sporulation. Thus, epidemiological information obtained for plant diseases (e.g. Coakley, 1988; van Maanen & Xu, 2003; Jeger, 2004) is pertinent in the context of a fungus–fungus association. Crop and forest microclimates are important epidemiological factors that influence plant disease, and their fluctuation is dependent upon plant architecture and height, canopy structure and management (Grimmond *et al.*, 2000; Sentelhas *et al.*, 2005; Heithecker & Halpern, 2006). Below a forest canopy, solar radiation and daytime air temperature are reduced, humidity is enhanced and wind speed is lowered and often decoupled from regional flow; these microclimatic differences are primary influences on understorey habitats and many biogeochemical processes (Grimmond *et al.*, 2000). Understanding how a biocontrol relationship develops under a specific canopy and management system is, therefore, important.

Unravelling microclimatic conditions that favour *Ts* sporulation is a starting point towards modelling the management of BCA application, aimed at forecasting periods in which both the development of disease and favourable conditions for the antagonist are expected. The objectives of this work were to (i) assess variation in key environmental parameters and determine the proportional effect of each on the onset of *Ts* sporulation in its mycoparasitic interaction with *Mp*, (ii) determine whether different heights of the cacao canopy exhibit different microclimates, and (iii) determine whether these canopy-related microclimates affect *Ts* sporulation and biocontrol activity. To achieve these goals, field experiments under usual environmental conditions of cacao farming in Bahia and employing previously selected *Ts* isolates (Carvalho, 2006) were conducted adjacent to a weather station, which recorded temperature in the air, leaf-litter and soil, air relative humidity (RH), aerial and leaf-litter broom moisture, solar radiation and wind speed above and below the canopy, and soil moisture.

Materials and methods

Experimental field

The meteorological and biological data related to this study were obtained from a typical cacao-farming area composed of 25-year-old cacao trees, at the Centro de Pesquisas do Cacau (CEPEC/CEPLAC) in the municipality of Ilhéus (south-eastern Bahia, Brazil, 14°31′S, 36°16′W, 55 m a.s.l.). These trees were grown on well drained soil with a ~2% slope, under the canopy of *Erythrina fusca*, a species normally used for shading. These shade trees were 15–20 m in height and spaced 24 × 24 m apart. The cacao trees were spaced 3 × 3 m apart, with their canopies occupying a vertical profile between the heights of 2·5 and 5·0 m, with most of the neighbouring trees having intermingling canopies.

Isolates and culture conditions

Five *Ts* isolates labelled as BA4 and BA8 from genetic group I, and BA29, BA66 and TVC from group II (de Souza *et al.*, 2006) were provided by Almirante Cacau Ltd for use in this study. These isolates had been previously studied for their biocontrol characteristics, in single-level understorey experiments (Carvalho, 2006). A 0·25-cm^2 filter-paper, containing previously grown and stored spores (Dhingra & Sinclair, 1985) from each isolate, was placed in a Petri dish containing potato-dextrose-agar (PDA) medium for incubation at room temperature (~25°C) for 14 days until sporulation (Sanogo *et al.*, 2002). Conidia were collected and thoroughly suspended in distilled water, with the

concentration adjusted to 10^7 spores mL^{-1} for application on brooms.

Preparation of brooms and application of *T. stromaticum* isolates

Segments of dead shoots and twigs (brooms) of several lengths, averaging 0·75 cm in diameter, were collected from cacao plants infected with *Mp* and tested for the presence of the pathogen and spontaneous sporulation of *Ts*. Segments 2 cm in length were cut from the ends of each broom and placed inside humid chambers consisting of small plastic bags containing wet filter paper. These segments were incubated at 25°C for 7 days; presence of white, cotton-like mycelium and white-to-green sporulation were the indicators of *Mp* and *Ts*, respectively. Only brooms containing *Mp* and free of *Ts* were used for the field experiments. The brooms were standardized to an average size of 20 cm in length and sprayed until run off (∼0·5 mL per broom) with spore suspension of *Ts*. To reduce the risk of contamination by *Ts* spores in the experimental area, the tagged-broom hanging and spraying procedures were performed in a separate washable area before placing them on the broomeries. Only a single-application treatment was used in the experiments.

Biological variables related to the *T. stromaticum*–*M. perniciosa* interaction

The variables used to assess the antagonistic *Ts*–*Mp* interaction were *Ts* sporulation at the end of the field trial (see experimental design, below) and biocontrol activity of the *Ts* isolates, gauged by the presence of *Mp* remaining in the broom. As only *Mp* basidiocarps could be readily seen in the field, the remaining viability of *Mp* in the brooms could only be assessed by detecting growth of its white mycelium, which required incubation in humid chambers after the field trial. *Mp* incidence and *Mp* severity were evaluated as the percentages of brooms and broom pieces per treatment, respectively, showing the white mycelium. Once a sporulating broom was identified in the field, it was removed from the experimental area, but kept under the same field conditions and at the same corresponding height, to prevent *Ts* cross-contamination with other brooms on the same hanger or between hangers (see experimental design, below). The dates and brooms on which *Ts* sporulation was detected were recorded. After the field-trial period, all sporulating and non-sporulating brooms were brought to the laboratory, cut into four pieces of identical size per broom, and placed into a humid chamber to incubate at room temperature for 7 days for the assessment of *Mp* incidence and severity. *Ts* sporulation was also used as the reference for analyses of the microclimatic parameters affecting the *Ts*–*Mp* interaction. The total number of sporulating brooms for all isolates at a given height and date was determined. All brooms in the experimental area were checked individually every 2–3 days, from the date they were treated with the *Ts* isolates until the end of the experiments.

Meteorological monitoring and analyses

Two experiments assessing the interaction between *Mp* and *Ts* isolates were conducted adjacent to a weather station CR23X (Campbell Scientific™). Measurements of key weather factors, in space and time, were obtained by positioning different types of sensors on a 6-m-tall scaffolding tower located in the cacao plantation mentioned above. The data acquisition system was based on previous designs by Strangeways (1972) and Tanner (1990), and was housed in a weatherproof corrosion-resistant aluminium box at a height of 1·75 m. Depending on the weather factor evaluated, the corresponding sensors were placed at two to four different heights in the cacao plantation. The meteorological parameters assessed were (i) above-canopy rainfall, using one Tipping Bucket gauge placed at 5·5 m; (ii) broom moisture, using four Fuel Moisture sensors (model CS505, Campbell Scientific) located in the leaf litter on the ground (0 m) and at 1·0, 2·5 and 5·5 m above the ground (previous analyses established that these sensors were able to mimic the actual fluctuation in broom moisture content with sufficient accuracy – R.A.C. Miranda, personal communication); (iii) air temperature at all four heights, (iv) air RH and solar radiation, using electronic sensors placed at the three above-ground heights, (v) wind speed, using sensors located at 1·0 and 5·5 m; and (vi) soil temperature and soil moisture, using probes set at a depth of 5 cm. The field trials were exact replicates of each other, the first being conducted from 13 September to 17 November 2004, and the second from 8 December 2004 to 8 March 2005, over a total of 66 and 91 days, respectively.

For analyses of the temporal profiles of meteorological variables, two-dimensional graphical plots were assembled using a spreadsheet-handling computer program (Microsoft EXCEL®) and the downloaded database, automatically generated in ASCII format by the software PC200W (Campbell Scientific Inc.™) connected to the weather station datalogger. Daily readings were used, with each variable being individually plotted along with an indication of *Ts* sporulation events. For most meteorological factors, two measurements per day, taken 12 h apart, were used: a night measurement at 04:00 h and a day measurement at 16:00 h. For solar radiation and wind speed, only measurements at 16:00 h were used, since the wind speed was nil or negligible at night (04:00 h) for the vast majority of days evaluated.

Experimental design

Special stands ('broomeries') were built to accommodate canopy-related treatments, allowing investigation of the *Ts*–*Mp* interaction at each of the four established heights. The overall design and characteristics of these structures for the two field experiments described above is shown in Fig. 1. The experiments were conducted in a completely

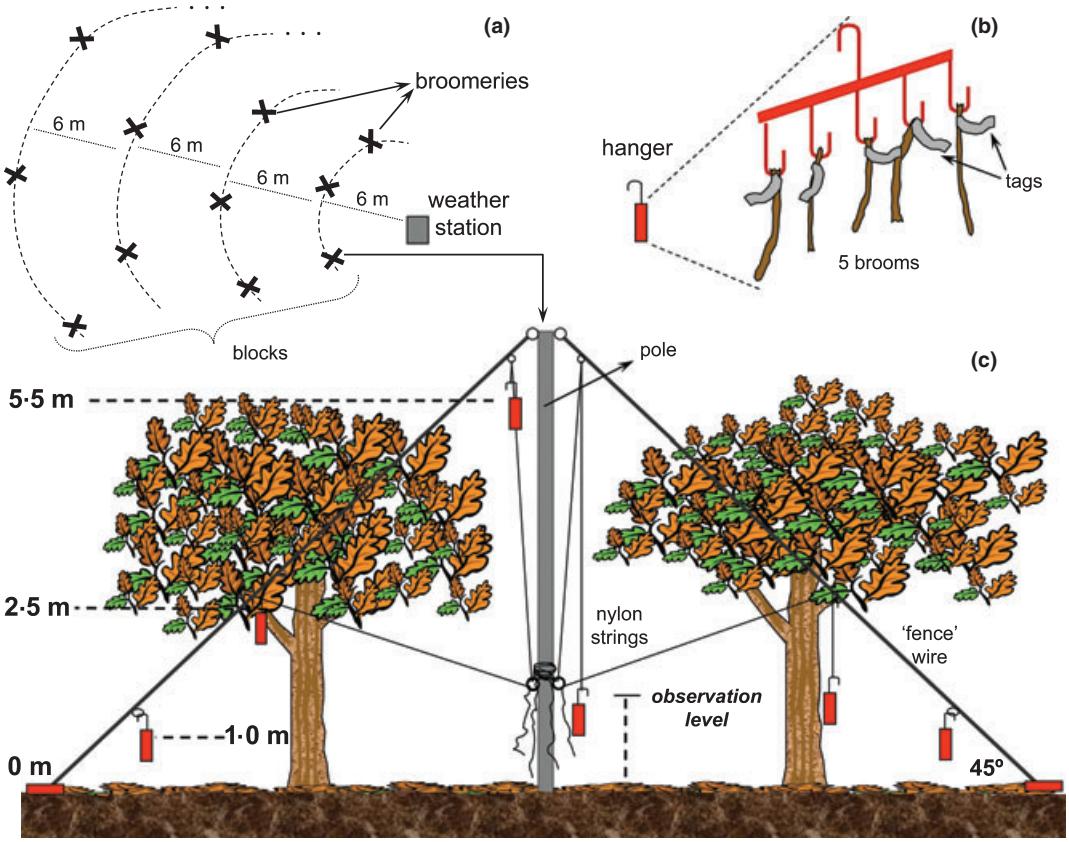

Figure 1 Schematic illustration of experimental design, hangers and stands ('broomeries') for cacao field experiments (see Materials and methods). (a) Top view of the experimental area, showing the spatial distribution of the broomeries and blocks radiating out from the weather station. (b) Enlarged view of a hanger with five individually tagged brooms, corresponding to a single experimental unit. (c) Lateral detailed view of a broomery in the field. Two 45º-inclined 'fence-like' straight wires were strongly attached to the ground and the pole's top, serving as support for both the whole broomery structure and the hangers. Hangers were placed at the indicated heights, using nylon strings to descend the treated brooms from 5·5 and 2·5 m to an observable level to record *Trichoderma stromaticum* sporulation. Each broomery held two isolate treatments at all heights, one on each side of the central pole.

randomized block design, with four blocks established as concentric rings radiating out from the weather station, on which the broomeries were positioned (Fig. 1a). The experimental unit for each treatment consisted of a horizontal hanger with five 20-cm individually tagged brooms, 15 cm apart from each other (Fig. 1b). A set of *Mp*-containing brooms treated with a single *Ts* isolate was hung in a straight 'fence-type' wire extending from 0 m (ground) up to 5·5 m, in an inclined arrangement (45º angle) to avoid cross-contamination of *Ts*-sporulating and non-sporulating brooms between heights; a single broomery held two isolate treatments, one on each side of the central pole (Fig. 1c). Similar structures were built away from the broomeries area, but under the same crop and canopy conditions, to store the sporulated brooms that were removed to prevent cross-contamination of the non-sporulated ones (see above). The distance of the first ring (block) from the station, and between rings, was 6 m, so that the fourth block was located at a 24-m radius from the station (Fig. 1a). This block arrangement was based upon the assumption that the weather measurements represent what occurs in a 30-m radius from the station (manufacturer's information). Each block contained six *Ts* treatments (five isolates plus a control with only distilled water) × four canopy levels (heights), giving a total of 24 treatments, which were randomly distributed among broomeries. After *Ts* application, the hangers were placed at the indicated heights (Fig. 1c). With this design, a total of 480 brooms (four blocks × six isolates × four heights = 96 hangers) were placed in the experimental area. Sporulation of *Ts* and presence of *Mp* were determined as the percentage of brooms scoring positive in each hanger (experimental unit). In order to compare canopy levels only, '*Ts*-sporulating' and 'non-*Ts*-sporulating' categories considered all isolates together, with *Mp* incidence calculated as; no. brooms with *Mp* / total no. brooms evaluated × 100, and severity as; no. broom pieces with *Mp* / total no. broom pieces evaluated × 100.

Statistical analyses

The results of *Ts* sporulation and *Mp* incidence at each height, from either the isolates combined or individually,

were first tested for normal distribution of data by the Lilliefors test (Lilliefors, 1967), with the percentages previously transformed by the square root of 'x + 1' (Steel & Torry, 1980). Since the null hypothesis for normality was rejected ($P < 0{\cdot}05$) for some samples, the non-parametric Kruskall-Wallis test was employed for each experiment separately. When the H value was significant, means comparison was performed by the Student-Newman-Keuls test ($P < 0{\cdot}10$).

To assess the association of aerial meteorological factors with sporulation, a logistic multiple regression analysis was performed, in which *Ts* sporulation was the dependent variable, and temperature (°C), relative humidity (%), broom moisture (%), solar radiation (Kw m^{-2}) and wind speed (m s^{-1}) were the independent variables. The absolute value (modulus) of the night-to-day difference for each of these five meteorological factors at each of the three above-ground canopy heights (1·0, 2·5 and 5·5 m) at a single date were considered for the analysis. The sporulation-positive values ('1') considered the readings at 2–3 days prior to the sporulation events, whereas the sporulation-negative values ('0') used readings from other dates randomly chosen along the experimental data profiles. The values used prior to sporulation events were chosen based on the graphical analysis of the temporal profiles of meteorological variables, as described above. A total of 20 positive and 20 negative data were used (n = 40, d.f. = 4) in the analysis. A high degree of multicolinearity is undesirable in this type of multiple regression model, as it produces unacceptable uncertainty (large variance) in the coefficient estimates. To assess such an effect in the multiple logistic regression, the independent variables were subjected to Pearson's linear correlation analysis; an R coefficient >0·7 led to the withdrawal of the air RH variable from the logistic regression (Tabachnik & Fidell, 2001). All statistics were performed using the open-access software BIOESTAT 5·0 (Ayres *et al*., 2007).

Results

Effects of meteorological factors on *T. stromaticum* sporulation

Considering the environmental influence previously identified on the interaction between *Ts* and *Mp* (Carvalho, 2006), the relationship between *Ts* sporulation events and environmental factors was monitored at different canopy heights. To assess the temporal fluctuation in aerial and leaf-litter broom moisture, air RH, air and soil temperature, solar radiation, wind speed and soil moisture, graphical plots were assembled with daily day and night readings (Fig. 2). For the aerial factors, reproducible pre-sporulation conditions were observed occurring 2–3 days prior to detection of sporulation. These conditions consisted of (i) above-canopy radiation intensity below the threshold for bright sunshine, i.e. 0·12 KW m^{-2}, based on World Meteorological Organization (WMO) standards, (ii) wind speed reduced to or near zero, (iii) moisture of brooms maintained above 30%, (iv) RH above 90%, and (v) temperature ranging between 20 and 26 °C, which matched the optimal temperature range previously obtained *in vitro* for *Ts* (Sanogo *et al*., 2002). For the latter three factors, a pattern of a reduced night-to-day variation (Δ) was also characteristic of these pre-sporulation periods. Although five different *Ts* isolates were applied, they essentially sporulated on the same dates, indicated by the vertical lines across the graphs (Fig. 2). Similar profiles of fluctuation in these factors were observed among canopy levels, with R values >0·98 after pairwise correlation analyses of data from each height. However, the magnitudes of the readings above and below the canopy were similar only for RH and temperature, which showed differences of less than 1% and 0·5°C, respectively, between heights at the same dates and time points. On the other hand, the night-to-day variation in broom moisture was higher at 5·5 m, and wind speed readings differed between above and below canopy by 1·0–1·5 m s^{-1} per day, with the higher values observed above the canopy. Not unexpectedly, a large difference in the magnitude of solar radiation values was observed between the levels above (5·5 m; Fig. 2) and below (2·5 and 1·0 m) the canopy, such that understorey radiation intensities never exceeded the bright sunshine threshold throughout the whole experimental period (data not shown).

As previously reported, brooms treated with *Ts* and left on the ground of a cacao plantation tend to be extensively covered with conidia (Sanogo *et al*., 2002). To verify the potential existence of conditions that could explain those higher levels of sporulation, the profiles of night-to-day fluctuation of moisture and temperature in relation to *Ts* sporulation were assessed on both the leaf-litter and soil (Fig. 2). When sporulation was observed, the dates were the same as for the above-ground heights. Not unexpectedly, night-to-day variations in temperature were lower on the ground than in the air temperature, ranging from 1 to 6°C for leaf litter and from 0·2 to 0·8°C for soil, in contrast to fluctuations averaging around 10°C above ground (Fig. 2). A lower night-to-day variation relative to above-ground heights was also observed for broom moisture at leaf-litter level, although the profiles were similar (Fig. 2). Specific environmental conditions preceding the sporulation events on the ground were also observed, characterized by increased intensity and duration of the moisture in the brooms (>30%), a night-to-day variation of less than 2°C for leaf-litter temperature, and a minimum soil-moisture content of 0·34 m^3 m^{-3} (Fig. 2). Despite differences in magnitude, these pre-sporulation patterns at the leaf-litter level were essentially similar to those above ground, although no specific pattern of fluctuation in soil temperature preceded sporulation (Fig. 2).

Microclimate-sporulation interaction

To determine the effect of microclimate on *Ts* sporulation, a multiple logistic regression analysis was carried out, using the absolute values of the night-to-day Δ for the

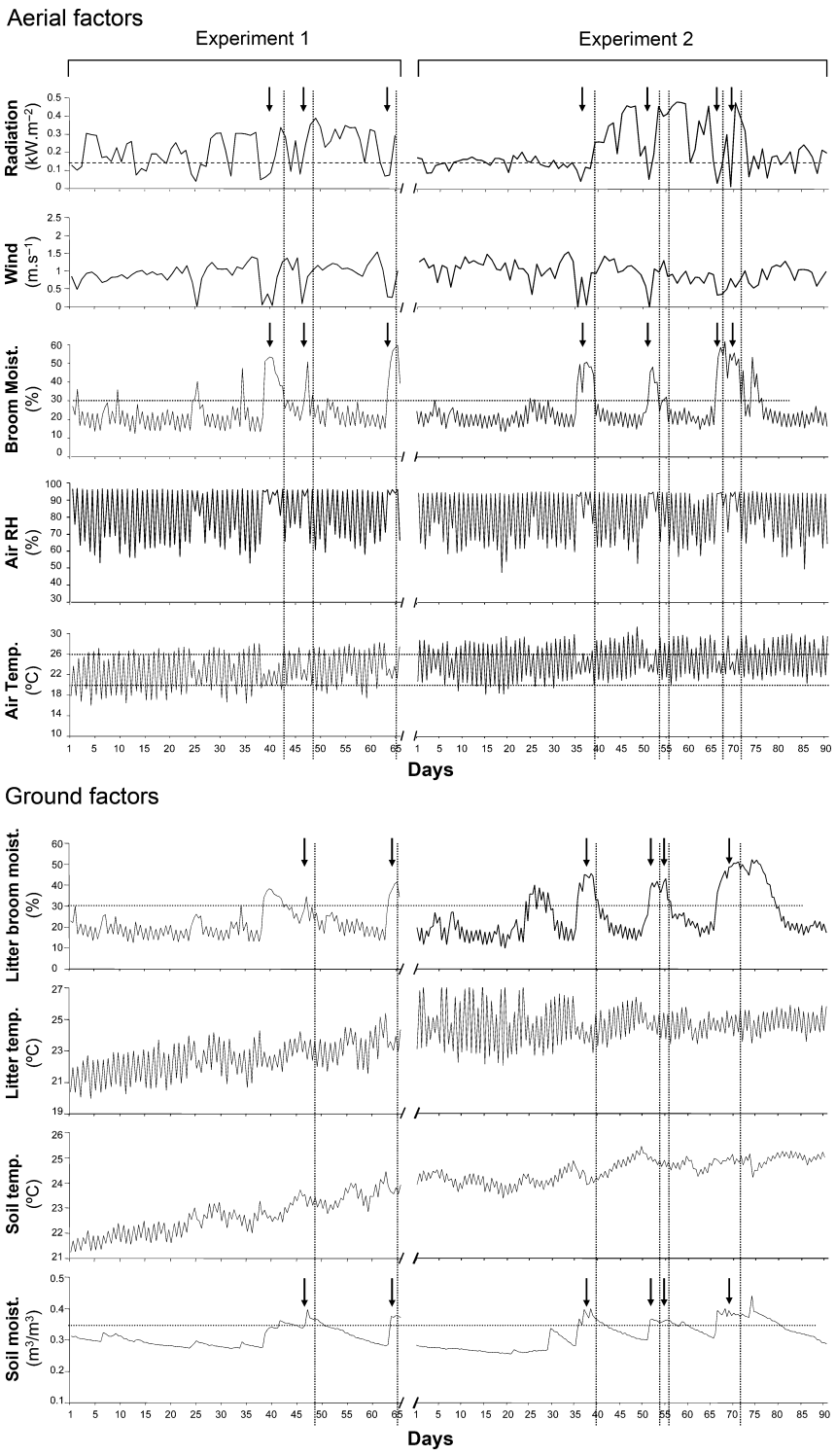

Figure 2 Graphical analysis of the temporal profiles of aerial and ground-related weather factors in relation to *Trichoderma stromaticum* sporulation under field conditions in cacao. Combined sporulation events for all isolates are indicated by vertical lines across all plots and the specific patterns of weather variation observed prior to sporulation events are indicated by arrows. All plots show night (04:00 h) and day (16:00 h) readings for each day, with the exception of solar radiation and wind speed, for which only 16:00-h readings at 5·5 m (above canopy) and at 1·0 m, respectively, are shown. For the aerial factors broom moisture, air humidity and air temperature, the average readings of the three above-ground canopy levels (1·0, 2·5 and 5·5 m) were plotted. Specific reference values presented in the text for broom moisture (aerial and ground), temperature, solar radiation and soil moisture are indicated by horizontal lines.

five above-ground variables assessed at each of the three canopy heights. The model including all five above-ground weather factors and the three above-ground canopy heights was significantly different from the one containing only the constant (χ^2 = 15·42, P = 0·0039), thereby indicating that the combined variables were capable of distinguishing *Ts*-sporulation from non-sporulation events. To avoid undesirable effects of multicollinearity among the variables in this type of analysis, pairwise linear correlations were performed. The threshold of an R coefficient >0·7 was adopted to decide whether a variable should be withdrawn from the regression model, as previously suggested (Tabachnik & Fidell, 2001). From the Pearson's linear correlation matrix produced by the analysis, the temperature–RH pair gave R = 0·974; the latter variable was therefore removed from the model, since another variable related to humidity (broom moisture), was still present. The results from the multiple logistic regression indicated that *Ts* sporulation can be adequately predicted by a specific set of environmental conditions, with a significant effect of wind speed among other components of the model (Table 1).

Table 1 Multiple logistic regression analysis of above-ground weather variables in relation to *Trichderma stromaticum* sporulation, at three canopy levels

Variable	Coefficient	*P*	Odds ratio
y-intercept	3·6247		
Broom moisture	0·0921	0·3587	1·0965
Temperature	–0·1648	0·3400	0·8481
Solar radiation	–0·1141	0·9841	0·8921
Wind speed	–3·8379	*0·0067*	0·0215

Differential behaviour of *T. stromaticum* isolates at different canopy levels

Based on the observed variation in the monitored weather factors, the effects of positioning the brooms at different canopy heights on both *Ts* sporulation and biocontrol activity, assessed as a function of the remaining *Mp* incidence and severity (Table 2), were investigated. Brooms with no *Ts* sporulation during the field trial showed a very high level of *Mp* incidence, ranging from 93 to 99%, whereas *Ts*-sporulating brooms displayed a dramatic drop in the incidence and severity of *Mp* at all canopy heights, with the former ranging from 7·3 to 28·1% and the latter from 5·1 to 13·8% (Table 2). At all canopy levels, *Mp* severity was maximum for non-sporulating brooms, but reduced for the *Ts*-sporulating ones (Table 2). Confirming an effect of canopy position, non-parametric statistical analysis of variance (Kruskall-Wallis test) indicated significant differences (H = 6·760) in *Ts* sporulation among heights (Table 2), with an inverse correlation between increasing amounts of *Ts*-sporulating brooms and decreasing heights of the canopy (R = 0·93). Considering all heights combined, *Mp* incidence was very significantly different between sporulating and non-sporulating brooms (H = 23·645).

Table 2 Percentages of *Trichoderma stromaticum (Ts)* sporulation in the field and presence of *Moniliophthora perniciosa (Mp)*, assessed by humid-chamber incubation after the second field trial, by cacao canopy height

Canopy levels[a]	Brooms (%)[b]	Presence of *Mp* (%)	
		Incidence[c]	Severity[d]
5·5 m (104 brooms)			
Ts-sporulating	16·3	20·8	11·5
non-*Ts*-sporulating	83·7	98·9	98·0
2·5 m (108 brooms)			
Ts-sporulating	27·3	28·1	13·8
non-*Ts*-sporulating	72·7	98·8	98·8
1·0 m (112 brooms)			
Ts-sporulating	30·5	7·3	6·1
non-*Ts*-sporulating	69·5	94·0	94·0
0·0 m (102 brooms)			
Ts-sporulating	41·0	10·3	5·1
non-*Ts*-sporulating	59·0	94·6	94·6

[a]Total no. of brooms in the experimental area at each height were considered; final no. of brooms used in the humid-chamber incubation (in parenthesis) were different because of losses during the field trial. Results from the two experiments showed the same trend (data from the second, longer experiment is presented).
[b]*Ts* sporulation (mean of four replicates) among heights was significantly different after Kruskall-Wallis test (P < 0·10), with data previously transformed by the square-root of x + 1.
[c]*Mp* incidences between *Ts*-sporulating and non-sporulating brooms (all levels combined; means of 16 replicates) were very significantly different after Kruskall-Wallis test (P << 0·001).
[d]Equal values for *Mp* incidence and severity in non-*Ts*-sporulating brooms indicate that, for those brooms with presence of *Mp* after the field trial, 100% of the broom pieces were colonized by the pathogen.

To investigate whether there was an isolate-specific response to the effects of microclimate variation, an analysis of sporulation for each *Ts* isolate at each canopy level was performed. The results showed that field sporulation was different among the isolates, with BA8 and BA4 (group I) generally sporulating more intensively than the others, a tendency that was fairly consistent at all heights; above canopy (at 5·5 m), isolate BA8 showed a significantly superior sporulation performance in both experiments (Table 3). In both experiments, individual and relative behaviour of isolates was similar overall, as observed by the narrow range of sporulation values per isolate/height between experiments, as well as by the average results from all levels combined. However, the statistical significance of differences in sporulation varied between heights and experiments, with no significant difference among all isolates being detected only in the first experiment at 2·5 m (Table 3). These results indicated an isolate-specific response to the different microclimates occurring at different canopy heights, in different experiments. Control brooms sprayed with water did not show *Ts* sporulation.

To investigate the effects of canopy-related microclimates on the *Ts–Mp* antagonistic interaction, *Mp*

Table 3 Sporulation of five *Trichoderma stromaticum (Ts)* isolates after two biocontrol field experiments.

		Ts isolate				
		Group II			Group I	
Height/experiment		TVC	BA29	BA66	BA8	BA4
5·5 m	exp. 1	1·00 ± 0·0**b**	1·00 ± 0·0**b**	1·90 ± 0·90**b**	7·40 ± 0·63**a**	3·60 ± 1·64**b**
	exp. 2	2·02 ± 1·02*b*	2·35 ± 1·35*b*	1·00 ± 0·0*b*	7·86 ± 1·18*a*	2·79 ± 1·03*b*
2·5 m	1	4·05 ± 1·79	1·90 ± 0·90	2·35 ± 1·35	6·05 ± 1·77	5·96 ± 1·86
	2	1·00 ± 0·0*c*	3·82 ± 0·95*bc*	2·92 ± 1·11*bc*	8·68 ± 1·37*a*	7·30 ± 0·95*ab*
1·0 m	1	1·00 ± 0·0**c**	3·25 ± 1·35**bc**	3·25 ± 1·35**bc**	5·05 ± 1·35**ab**	7·56 ± 1·13**a**
	2	2·35 ± 1·35*c*	1·90 ± 0·90*c*	2·54 ± 1·54*bc*	8·81 ± 0·86*a*	6·78 ± 0·91*ab*
0 m	1	5·04 ± 0·46**c**	5·95 ± 0·46**bc**	3·25 ± 1·35**c**	8·93 ± 0·65**a**	8·58 ± 0·90**ab**
	2	2·02 ± 1·02*c*	5·39 ± 0·81*bc*	3·95 ± 1·86*c*	8·86 ± 0·08*ab*	9·79 ± 0·26*a*
Average of all heights	1	2·77 ± 0·62**b**	3·02 ± 0·61**b**	2·68 ± 0·58**b**	6·86 ± 0·66**a**	6·43 ± 0·80**a**
	2	1·85 ± 0·46	3·36 ± 0·58	2·60 ± 0·65	8·55 ± 0·46	6·67 ± 0·75

Values in the table are square-root-transformed mean±SE percentages of *Ts* sporulation (see Materials and methods). The two extreme values of 9·79 and 1·00 in the table correspond to 94·9% and 0·0% sporulation, respectively. Control brooms sprayed only with water showed null *Ts* sporulation.

Statistics performed by a non-parametric analysis of variance (Kruskall-Wallis test, $P < 0{\cdot}05$) on a per-height/experiment basis; *H* value was not significant in experiment 1 at the 2·5-m level. Means in rows with same letters do not significantly differ by the Student-Newman-Keuls test ($P < 0{\cdot}10$). Significance letters for the means of all levels (underlined) were the same for both experiments.

incidence was also evaluated at the four canopy levels, on a per-isolate basis (Fig. 3). Results showed an isolate-specific response to different heights in terms of pathogen presence, and confirmed a tendency previously found of a negative association between *Ts* sporulation and *Mp* incidence (Loguercio *et al.*, 2009), independent of canopy height. The lower the amount of *Ts* sporulation for all isolates, the greater the presence of the pathogen, at all heights, with a generally higher efficiency for group-I isolates (Fig. 3). With the exception of ground level, at which BA4 was more efficient in decreasing *Mp* incidence, isolate BA8 showed a higher control activity at all levels above ground, although it was significantly different from BA4 only above the canopy; BA8 was also not significantly different than BA29 (group II) at 2·5 m (Fig. 3). *Mp* incidence and severity was maximal for brooms sprayed with water (controls). Production of *Mp* basidiocarps during field trials and humid-chamber incubations was also assessed. Despite the fact that very few of these structures were found, mostly on the control treatments, the pattern was erratic and prevented any further analysis. Moreover, occasional production of basidiocarps during humid-chamber incubation was found only for the *Ts*-non-sporulating brooms.

Discussion

Inconsistencies in the activity of BCAs, mostly influenced by effects of uncontrolled variation in the environment (Elad, 2003; Assante *et al.*, 2004; Benítez et al., 2004), limit the wider use of this method in commercial crops. For understorey crops such as cacao, it has been observed that the microclimate within the crop may be quite different from standard weather conditions (Bonaparte & Ampofo, 1975), and that the structure and dynamics of this agrosystem exert some influence on the microclimate, thereby affecting cacao diseases (Monteith & Butler, 1979; Butler, 1980; Rudgard & Butler, 1987). This study assessed the combined effects of several microclimatic factors on the sporulation of *Ts* isolates during their antagonistic interaction with the *Mp*, the causal agent of the witches' broom disease in cacao.

Simultaneous data collection and analysis of environmental factors revealed a specific set of conditions that consistently preceded the onset of BCA sporulation (Fig. 2). This 'pre-sporulation' microclimate consisted of reduced night-to-day fluctuations in temperature, RH and broom moisture, which were maintained at levels previously observed *in vitro* and in the field (Sanogo *et al.*, 2002; Pomella, unpublished). In addition, radiation intensity above the canopy was below the threshold for full sunshine and wind speed was close to zero (Fig. 2). Altogether, these conditions suggest that *Ts* sporulation is unlikely to occur under bright sunlight, in agreement with many other studies showing that drier air caused by high sunlight and wind inhibits fungal sporulation (e.g. Suzuki, 1975; O'Neill *et al.*, 1997). Since solar heating generates a localized decrease in air pressure, thus establishing gradients that induce wind (Tubelis & Nascimento, 1988), a coordinated decrease in these factors is not unexpected and probably explains the maintenance of moisture at higher levels and lower night-to-day variations in temperature during pre-sporulation periods (Fig. 2). This was supported by the multiple logistic regression analysis, as a statistically significant effect of wind speed was observed (Table 1). In models dealing with plant diseases, the relevance of assessing these weather factors simultaneously and the importance of wind in altering moisture levels have been reported (e.g. Pietravalle *et al.*, 2003; Chakraborty *et al.*, 2004). The

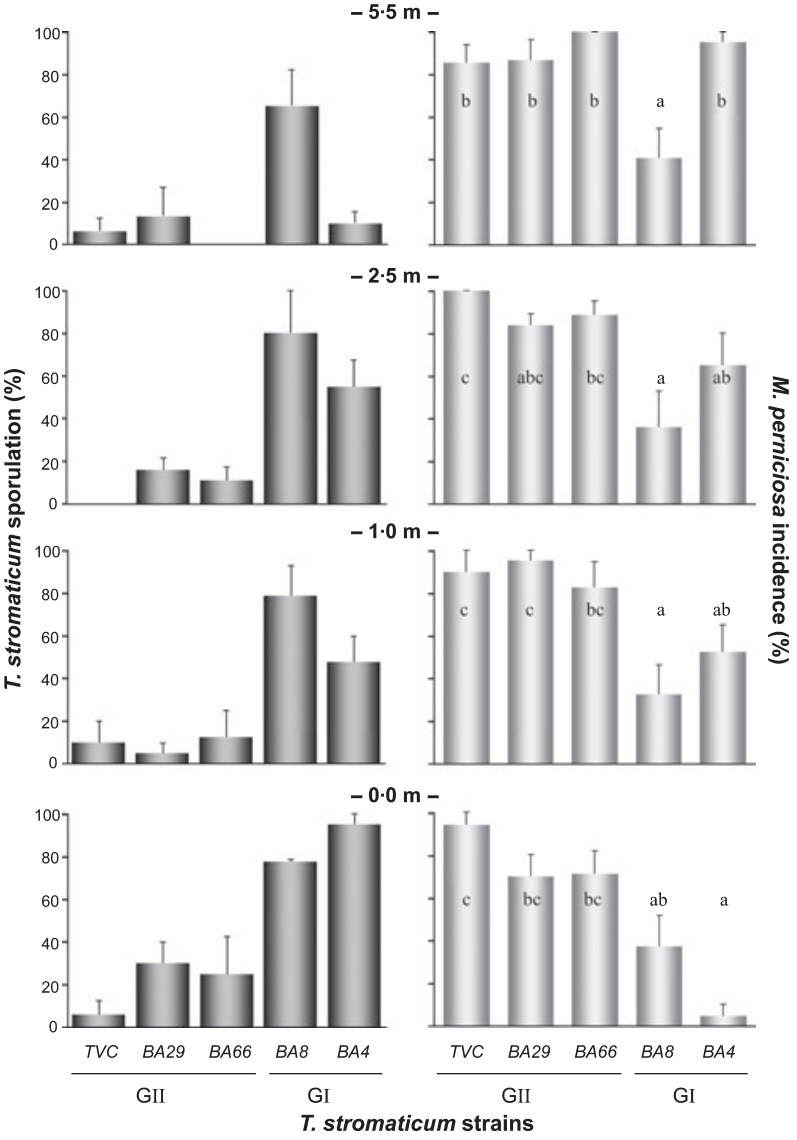

Figure 3 Average sporulation and *Moniliophthorn perniciosa* incidence for five *Trichoderma stromaticum* isolates at different cacao canopy levels (0, 1·0, 2·5 and 5·5 m). Graphs on the left refer to *Ts* sporulation and those on the right to the corresponding *Mp* incidence. Canopy height is indicated above each pair of graphs. The five *Ts* isolates used (italicized) of the corresponding genetic groups (GII and GI) are indicated at the bottom for all canopy levels. Error bars correspond to four replicates per isolate at each height (see Materials and methods). Brooms without *Ts* inoculation (control treatments) showed no sporulation and 90–100% *Mp* incidence. Non-parametric analysis of variance (Kruskall-Wallis test, $P < 0{\cdot}05$) was performed for each height; letters on the right-hand graphs indicate statistical differences (Student-Newman-Keuls test, $P < 0{\cdot}10$) among *Ts* isolates in the control of *Mp*. The experiment was performed twice, with similar results (see Table 3). Isolate TVC is the active ingredient of the commercial biocontrol product Tricovab (see Introduction).

results of the present study also provided better indications of where, in the vertical profile of a shaded cacao crop, microclimatic variables are worth measuring. Air temperature and RH were the same across the canopy profile, suggesting that air movements in the plantation tend to rapidly homogenize these variables (Miranda *et al.*, 1994). Therefore, their assessment at different heights is unnecessary. Variation in solar radiation below the canopy is not relevant, since conditions of full sunshine never occurred at the heights of 1·0 and 2·5 m (data not shown). However, assessment of sunlight above the canopy seems to be critical, as suggested by the multiple regression model (Table 1). Moreover, full direct sunlight may interfere with *Ts* sporulation by possibly placing selective pressure upon differentially tolerant isolates (Ten Hoopen *et al.*, 2003).

Periods of increased rainfall during the experiments essentially matched the decreased night-to-day fluctuation of temperature, RH and broom moisture (data not shown). This confirmed a previous report showing that rapid drops in temperature and increases in RH at all heights can occur 1 or 2 h after rains that sufficiently wet the cacao canopy (Miranda *et al.*, 1994). Different types and intensities of rainfall do not have the same partition through the cacao canopy, thereby creating distinct variations in moisture levels (Miranda, 1994). Since solar radiation also decreases during rain because of cloudiness, the combined effects of these two factors could presumably account for the patterns of variation observed for temperature and humidity in this environment (Fig. 2).

Considering that (i) night-to-day variation in litter temperature was generally lower than that in the air, (ii) temperature close to the ground oscillated mostly around the best values for *Ts* sporulation (i.e. between 22 and 26°C), and (iii) night-to-day variation in broom moisture at leaf-litter level was the lowest (data not shown), it is

reasonable to assume that the conditions on the ground are more suitable for *Ts* growth and development than those elsewhere, thereby explaining the previously reported tendency for higher sporulation on the ground (Sanogo *et al.*, 2002; Costa *et al.*, 2006). Furthermore, a minimal soil-moisture content of 0·34 $m^3 m^{-3}$ was shown to be an important reference in a cacao plantation, since this parameter preceded 100% of *Ts* sporulation events on the ground (Fig. 2).

Survival, growth, reproduction, abundance and overall biocontrol efficiency are fungal parameters that respond to environmental factors (e.g. Dik & Elad, 1999; Kredics *et al.*, 2000, 2004; Sanogo *et al.*, 2002; Ten Hoopen *et al.*, 2003). In the system studied here, the assessment of microclimatic effects on *Ts* sporulation was effective in gauging the biology–microclimate interaction for this specific mycoparasitic relationship. The production of *Ts* conidia on brooms not only represented a measure of an isolate's adaptation and establishment, but also served as a direct indicator of antagonism against *Mp* (Table 2), as *Ts* sporulation has shown to be negatively correlated with emergence of white *Mp* mycelia from treated brooms (Loguercio *et al.*, 2009). Because of the experimental design adopted adjacent to the weather station (Fig. 1), microclimate had to be considered the same at each height. Hence, it was fair to assume that all isolates were affected by a canopy-related microclimate in the same manner. The fact that sporulation occurred simultaneously for all isolates (Fig. 2) indicated that not only was the approach of assessing overall *Ts* sporulation and biocontrol effect as a function of canopy position appropriate, but also that at least part of the *Ts* biology–microclimate interaction was of a general nature. Nevertheless, a significant positional effect was detected for *Ts* sporulation across the canopy (Table 2) and the isolates behaved differently among heights and experiments in terms of sporulation and biocontrol activity (Table 3, Fig. 3). Since the five tested isolates belong to two distinct genetic groups (de Souza *et al.*, 2006), these results suggest the existence of isolate-specific responses to weather variation at the level of the different microclimates occurring above and below canopy, at different times of the year. This conclusion is in agreement with previous results obtained for a collection of 63 *Ts* isolates tested in the field, followed by four validation experiments using the same set of isolates studied here, which were all studied at a single level below the cacao canopy, but in different periods of the year (Loguercio *et al.*, 2009). Moreover, similar results to those presented here were obtained in further multiple-canopy-level experiments conducted under the same conditions (data not shown).

Variability in microclimates of different crop canopies is caused by the interaction of weather and plant height, architecture and structure (Sentelhas *et al.*, 2005), indicating the importance of understanding how a given biocontrol relationship occurs under specific canopy patterns and management systems. Modelling of plant disease epidemiology based on relationships with environmental factors provides the means for forecasting epidemics (van Maanen & Xu, 2003), important for integrated disease management strategies (Jeger, 2004). Multiple regression models were shown to be effective in explaining microclimate variation in forest environments (Heithecker & Halpern, 2006), and in providing reference points for more sophisticated and complex models, capable of offering sufficiently accurate predictions to sites other than those that generated the data (Chakraborty *et al.*, 2004). A similar perspective can be taken with biocontrol agents. For example, the knowledge of weather effects on specific BCAs can be combined with their specific biological requirements to improve biocontrol formulations. In addition, predictive models can be developed to provide more cost-effective application strategies for BCAs in terms of season and frequency. Similarly, screening procedures for identification of effective biocontrol isolates could include the systematic assessment of local fluctuations in weather conditions, so that the interpretation of the results would be more accurate. Finally, understanding differential sensitivities of BCAs to characteristic crop microclimates would help to reduce inconsistencies in their use, as more knowledgeable strategies can be designed (Hjeljord & Tronsmo, 1998). For instance, since trends indicated a drier, more variable microclimate above the cacao canopy, *Ts* isolate BA8 (or any other with a similar behaviour) could be applied to brooms in the top portion of the canopy, or in periods when less humidity is expected (both in the air and in the brooms). This application may be either alone or in combination with other isolates showing adequate antagonism at other levels below the canopy and/or found to have synergistic controlling effects with BA8. Further studies are certainly warranted to investigate these possibilities in depth. The development of strategies for efficient 'biological broom pruning', with the use of more suitable *Ts* isolates for this purpose, is promising.

Under the antagonistic conditions of this study, the results showed that *Ts* sporulation is not only influenced by the natural limits of broom moisture, air temperature and RH values within which the fungus can live (e.g. Sanogo *et al.*, 2002; Carvalho, 2006), but, more importantly, by specific weather patterns and how they vary during the day. Low night-to-day variation in key environmental parameters is probably necessary for a sustained metabolism and physiology, which in turn, allows the completion of the BCA's life cycle as a hyperparasite (indicated by sporulation). Thus, what probably prevents more frequent canopy-related *Ts* sporulation under field conditions is high daily night-to-day variation in moisture and temperature (Fig. 2), especially as most *Trichoderma* isolates are thermally mesophilic and do not tolerate high osmotic potentials caused by drying conditions in the environment, which impose some of the recognized limitations for their use as biofungicides (Kredics *et al.*, 2003). This is particularly relevant in the context of witches' broom disease of cacao, as previous studies have shown that *Mp* is probably more adapted to water stress than *Ts* and copes better with the higher daily fluctuations in moisture found in aerial brooms than those on the

leaf-litter (Griffith *et al*., 1994). As similarly discussed for other systems (e.g. Colhoun, 1973; Coakley, 1988; Grimmond *et al*., 2000; Elad, 2003; Chakraborty *et al*., 2004; Sentelhas *et al*., 2005), the present study suggests that simultaneous observation of relevant microclimatic variables, key biological parameters and canopy-related aspects can provide valuable inputs for biocontrol-related variables in mathematical modelling, thereby leading to predictive strategies for more efficient integrated disease management in shaded cacao plantations.

Acknowledgements

The authors are thankful to Mr Carlos Alberto Leal Santos, Antônio Gomes Galvão Filho and MSc Aítala Carvalho de Carvalho for their technical assistance, to Dr Maurício Cetra for statistical advice, and to Drs Lyndell Meinhardt, Alison Stewart and Mark Braithwaite for the critical review of the manuscript. The research was funded by USDA-ARS/CEPLAC Cooperative SCA Agreement no. 58-1275-3-F101, PADCT-CNPq (Brazil) and MARS-Almirante Cacau. LSS was supported by a M.Sc. scholarship from FAPESB (Bahia, Brazil).

References

Aime MC, Phillips-Mora W, 2005. The causal agents of witches' broom and frosty pod rot of cacao (chocolate, *Theobroma cacao*) form a new lineage of Marasmiaceae. *Mycologia* **97**, 1012–22.

Andebhran T, Figueira A, Yamada MM, Cascardo J, Furtek DB, 1999. Molecular fingerprinting suggests two primary outbreaks of witches' broom disease (*Crinipellis perniciosa*) of *Theobroma cacao* in Bahia, Brazil. *European Journal of Plant Pathology* **105**, 167–75.

Assante G, Maffi D, Saracchi M, Farina G, Moricca S, Ragazzi A, 2004. Histological studies on the mycoparasitism of *Cladosporium tenuissimum* on urediniospores of *Uromyces appendiculatus*. *Mycological Research* **108**, 170–82.

Ayres M, Ayres M Jr, Ayres DL, dos Santos AAS, 2007. *BioEstat – Aplicações Estatísticas nas Áreas de Ciências Biomédicas*. Belém, PA, Brazil: Sociedade Civil Mamirauá/MCT-CNPq (http://www.mamiraua.org.br).

Bastos CN, 1996. Mycoparasitic nature of the antagonism between *Trichoderma viride* and *Crinipellis perniciosa*. *Fitopatologia Brasileira* **21**, 50–4.

Bastos CN, 2000. *Trichoderma stromaticum* sp. nov. na produção de basidiomas e infecções de ramos e almofadas florais do cacaueiro por *Crinipellis perniciosa*. *Agrotrópica* **12**, 59–62.

Benítez T, Rincón AM, Limón MC, Codón AC, 2004. Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology* **7**, 249–60.

Bonaparte EENA, Ampofo S, 1975. The cacao microclimate. In: *Proceedings of the 5th International Cacao Research Conference*, Ibadan, Nigeria. 210–5.

Butler DR, 1980. Dew and thermal lag: measurements and an estimate of wetness duration on cocoa pods. *Quarterly Journal of the Royal Meteorological Society* **106**, 539–50.

Carvalho AC, 2006. *Bioprospection of* Trichoderma stromaticum *Isolates for the Biological Control of the Witches' Broom Disease of the Cocoa Tree*. Ilhéus, BA, Brazil: State University of Santa Cruz/UESC, MSc thesis.

Chakraborty S, Ghosh R, Ghosh M, Fernandes CD, Charchar MJ, Kelemu S, 2004. Weather-based prediction of anthracnose severity using artificial neural network models. *Plant Pathology* **53**, 375–86.

Coakley SM, 1988. Variation in climate and prediction of disease in plants. *Annual Review of Phytopathology* **26**, 163–81.

Colhoun J, 1973. Effects of environmental factors on plant disease. *Annual Review of Phytopathology* **11**, 343–64.

Costa JCB, Bezerra JL, Veloso JLM, Niella GR, Bastos CN, 2006. Controle biológico da vassoura-de-bruxa do cacaueiro. In: Venzon M, Paula Júnior TJ, Pallini A, eds. *Tecnologias Alternativas para o Controle de Pragas e Doenças*. Viçosa-MG, Brazil: EPAMIG, 25–47.

Dhingra OD, Sinclair JB, 1985. *Basic Plant Pathology Methods*. Boca Raton, FL, USA: CRC Press.

Dik AJ, Elad Y, 1999. Comparison of antagonists of *Botrytis cinerea* in greenhouse-grown cucumber and tomato under different climatic conditions. *European Journal of Plant Pathology* **105**, 123–37.

Donald PF, 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology* **18**, 17–37.

Elad Y, 2003. Biocontrol of foliar pathogens: mechanisms and application. *Communications in Agriculture and Applied Biological Sciences* **68**, 17–24.

Faria D, Laps RR, Baumgarten J, Cetra M, 2006. Bat and bird assemblages from forests and shade cacao plantations in two contrasting landscapes in the Atlantic rainforest of southern Bahia, Brazil. *Biodiversity and Conservation* **15**, 587–612.

Griffith GW, Bravo-Velasquez E, Wilson FJ, Lewis DM, Hedger JN, 1994. Autecology and evolution of the witches' broom pathogen (*Crinipellis perniciosa*) of cocoa. In: Blakeman JP, Williamson B, eds. *The Ecology of Plant Pathogens*. Wallingford, UK: CAB International, 245–67.

Grimmond CSB, Robeson SM, Schoof JT, 2000. Spatial variability of micro-climatic conditions within a mid-latitude deciduous forest. *Climate Research* **15**, 137–49.

Heithecker TD, Halpern CB, 2006. Variation in microclimate associated with dispersed-retention harvests in coniferous forests of western Washington. *Forest Ecology and Management* **226**, 60–71.

Hjeljord L, Tronsmo A, 1998. *Trichoderma* and *Gliocladium* in biological control: an overview. In: Harman GE, Kubicek CP, eds. Trichoderma *and* Gliocladium – *Enzymes, Biological Control and Commercial Applications*, vol. 2. London, UK: Taylor & Francis, 131–51.

Howell CR, 1998. The role of antibiosis in biocontrol. In: Harman GE, Kubicek CP, eds. Trichoderma *and* Gliocladium – *Enzymes, Biological Control and Commercial Applications*, vol. 2. London, UK: Taylor & Francis, 173–84.

Jeger MJ, 2004. Analysis of disease progress as a basis for evaluating disease management practices. *Annual Review of Phytopathology* **42**, 61–82.

Johns ND, 1999. Conservation in Brazil's chocolate forest: the unlikely persistence of the traditional cocoa agroecosystem. *Environmental Management* **23**, 31–47.

Kredics L, Antal Z, Manczinger L, 2000. Influence of water potential on growth, enzyme secretion and *in vitro* enzyme

activities of *Trichoderma harzianum* at different temperatures. *Current Microbiology* **40**, 310–4.

Kredics L, Antal Z, Manczinger L, Szekeres A, Kevei F, Nagy E, 2003. Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. *Food Technology and Biotechnology* **41**, 37–42.

Kredics L, Manczinger L, Antal Z *et al*., 2004. *In vitro* water activity and pH dependence of mycelial growth and extracellular enzyme activities of *Trichoderma* strains with biocontrol potential. *Journal of Applied Microbiology* **96**, 491–8.

Lilliefors H, 1967. On the Kolmogorov-Smirnov test for normality with mean and variance unknown. *Journal of the American Statistical Association* **62**, 399–402.

Loguercio LL, Carvalho AC, Niella GR, de Souza JT, Pomella AWV, 2009. Selection of *Trichoderma stromaticum* isolates for efficient biological control of the witches' broom disease in cacao. *Biological Control*. Doi: 10.1016/j.biocontrol.2009.06.005.

Lopes VL, Monteiro WR, Pires JL, da Rocha JB, Pinto LRM, 2003. On farm selection for witches' broom resistance in Bahia, Brazil – a historical retrospective. In: *14th International Cocoa Research Conference, Accra, Ghana*, vol. 2. Lagos, Nigeria: COPAL, 1001–6.

van Maanen A, Xu XM, 2003. Modelling plant disease epidemics. *European Journal of Plant Pathology* **109**, 669–82.

Miranda RAC, 1994. Partitioning of rainfall in a cocoa (*Theobroma cacao* L.) plantation. *Hydrological Processes* **8**, 351–8.

Miranda RAC, Milde LCE, Bichara AL, Cornell SE, 1994. Daily characterization of air temperature and relative humidity profiles in a cocoa plantation. *Pesquisa Agropecuaria Brasileira* **29**, 345–53.

Monteith JL, Butler DR, 1979. Dew and thermal lag: a model for cocoa pods. *Quarterly Journal of the Royal Meteorological Society* **105**, 207–15.

O'Neill TM, Shtienberg D, Elad Y, 1997. Effect of some host and microclimate factors on infection of tomato stems by *Botrytis cinerea*. *Plant Disease* **81**, 36–40.

Pereira JL, Almeida LCC, Santos SM, 1996. Witches' broom disease of cocoa in Bahia: attempts at eradication and containment. *Crop Protection* **15**, 743–52.

Pietravalle S, Shaw MW, Parker SR, van den Bosch F, 2003. Modeling of relationships between weather and *Septoria tritici* epidemics on winter wheat: a critical approach. *Phytopathology* **93**, 1329–39.

Pomella AWV, de Souza JT, Niella GR *et al*., 2007. The use of *Trichoderma stromaticum* in the management of witches' broom disease of cacao in Bahia State, Brazil. In: Vincent C, Goettel M, Lazarovits G, eds. *Biological Control: A Global Perspective – Case Studies from Around the World*. Wallingford, UK: CABI Publishing, 210–7.

Purdy LH, Schmidt RA, 1996. Status of cacao witches' broom: biology, epidemiology, and management. *Annual Review of Phytopathology* **34**, 573–94.

Rudgard SA, Butler DR, 1987. Witches' broom disease on cocoa in Rondônia, Brasil: pod infection in relation to pod susceptibility wetness, inoculum and phytosanitation. *Plant Pathology* **36**, 512–22.

Samuels GJ, Pardo-Schultheiss R, Hebbar PK *et al*., 2000. *Trichoderma stromaticum* sp. nov., a parasite of the cacao witches broom pathogen. *Mycological Research* **104**, 760–4.

Sanogo S, Pomella A, Hebbar PK *et al*., 2002. Production and germination of conidia of *Trichoderma stromaticum*, a mycoparasite of *Crinipellis perniciosa* on cacao. *Phytopathology* **92**, 1032–7.

Santos LS, 2005. *Antagonistic interaction* Trichoderma stromaticum–Crinipellis perniciosa*: Climate Effects in the Biocontrol of Cacao Witches' Broom and Development of Microsatelite Markers*. Ilhéus-BA, Brazil: State University of Santa Cruz/UESC. MSc thesis.

Sentelhas PC, Gillespie TJ, Batzer JC *et al*., 2005. Spatial variability of leaf wetness duration in different crop canopies. *International Journal of Biometeorology* **49**, 363–70.

de Souza JT, Pomella AWV, Bowers J, Pirovani CP, Loguercio LL, Hebbar P, 2006. Genetic and biological diversity of *Trichoderma stromaticum*, a mycoparasite of the cacao witches' broom pathogen. *Phytopathology* **96**, 61–7.

Steel RG, Torry JH, 1980. *Principles and Procedures of Statistics*, 2nd edn. New York, USA: McGraw-Hill Book Co.

Steyaert JM, Ridgway HJ, Elad Y, Stewart A, 2003. Genetic basis of mycoparasitism: a mechanism of biological control by species of *Trichoderma*. *New Zealand Journal of Crop and Horticultural Science* **31**, 281–91.

Strangeways IC, 1972. Automatic weather station for network operation. *Weather* **27**, 403–8.

Suzuki H, 1975. Meteorological factors in the epidemiology of rice blast. *Annual Review of Phytopathology* **13**, 239–56.

Tabachnik BG, Fidell LS, 2001. *Using Multivariate Statistics*. Boston, MA, USA: Allyn and Bacon.

Tanner BD, 1990. Automatic weather station. *Remote Sensing Reviews* **5**, 73–98.

Ten Hoopen GM, Rees R, Aisa P, Stirrup T, Krauss U, 2003. Population dynamics of epiphytic mycoparasites of the genera *Clonostachys* and *Fusarium* for the biocontrol of black pod (*Phytophthora palmivora*) and moniliasis (*Moniliophthora roreri*) on cocoa (*Theobroma cacao*). *Mycological Research* **107**, 587–96.

Tubelis A, Nascimento FJL, 1988. *Meteorologia Descritiva – Fundamentos e Aplicações Brasileiras*. São Paulo, SP, Brazil: Nobel.